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The identification of multiple SNP markers for scald resistance in spring barley through restriction-site associated sequencing

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Abstract Leaf scald caused by *Rhynchosporium commune* (formerly *R. secalis*) is an important fungal disease in barley (*Hordeum vulgare*) in western Canada. Pyramiding multiple scald resistance qualitative trait loci (QTL) is considered an effective strategy for breeding durable scald resistance into barley. The objective of this study was to map QTLs with restriction site-associated (RAD) markers and identify single nucleotide polymorphism (SNP) markers for scald resistance. Two recombinant inbred line (RIL) populations (crosses Harrington/Seebe and Seebe/Shyri) with good resistance to local scald isolates were genotyped by RAD sequencing (RAD-seq). A total of 4203 SNPs were obtained from the RADseq analysis of 162 F7 RILs. Inclusive composite interval mapping identified multiple major QTLs for scald resistance on chromosomes 3H, 4H, and 5H in Shyri, and 2H and 6H in Seebe. SNP markers from Shyri were located in the same region as the previously reported 3H QTL Rrs1. Several SNP markers from Seebe clustered on chromosome 6H near a major scald resistance QTL. The 6H QTL appeared effective for both adult plant resistance and seedling resistance, and explained up to 70.9% of the phenotypic variation among the sequenced lines. A tightly linked SNP for the major 6H QTL was converted into an allele specific PCR marker. The QTLs and their genetic markers found in this study will be useful in barley breeding for the selection of resistance to barley leaf scald.

Keywords Barley · Scald-resistance · Restriction-site associated DNA sequencing (RADseq) · Single-nucleotide polymorphism (SNP) · Marker assisted selection (MAS) · Qualitative trait loci (QTL)

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Introduction

Leaf scald, caused by the fungus *Rhynchosporium commune* (formerly *R. secalis*), is a major barley disease in western Canada as well as other barley growing regions worldwide (Zhan et al. 2008). Controlling scald by using resistant cultivars is generally effective, but resistance can be overcome by the diverse virulence of *R. commune* populations at different locations (Xi et al. 2003b). However, durable resistance to barley scald can be obtained by pyramiding multiple resistance genes from different sources into one variety (Cséleny et al. 1998). Evaluating barley leaf scald resistance in the greenhouse or field is labour intensive, time consuming, and

does not provided information about the genetic origin of the specific scald resistance. Phenotypic testing can also confuse disease resistance with disease escape. Therefore, breeders could greatly benefit by the development of reliable markers for scald resistance to better characterize the genetics behind the resistance, however in order to accomplish this task breeders need reliable molecular markers to identify and follow the transfer of specific resistance genes.

From previous studies on barley resistance to *R. commune*, up to 43 QTLs and seven genes have been reported (Wang et al. 2014b). Rrs1 on the long arm of chromosome 3H is a complex locus representing either different tightly linked genes or multiple alleles of a single gene (Bjørnstad et al. 2002). Resistance QTLs mapped on 7H include Rrs2, Rrs12 and Rrs15 (Genger et al. 2005). Resistance QTL, Rrs3 was identified on chromosome 4H by Bjørnstad et al. (2002). The Rrs4 from the Ethiopian landrace Nigrinudum was located on chromosome 3HL over 20cM away from Rrs1 (Patil et al. 2003) and Rrs13 was mapped on chromosome 6H (Abbott et al. 1995). From Iranian and Turkish wild barley, *H. vulgare* L. ssp *spontaneum* (C. Koch) Thell, Rrs14 was introduced and mapped on chromosome 1H (Garvin et al. 2000). While, the Rrs16 QTL originating from wild barley species *H. bulbosum* has been mapped on chromosome 4HS (Pickering et al. 2006) and several minor scald QTLs were reported on chromosome 2H and chromosome 5H by Looseley et al. (2012) along with major QTLs on chromosome 3H and chromosome 7H.

Several molecular markers have been previously reported for scald resistance QTLs. On the 1H, seed storage proteins (hordeins), Hor1 and Hor2 link to the Rrs14 gene (Garvin et al. 2000). Simple sequence repeat (SSR) markers GBM1281 and GemS13 are linked to a major scald resistance QTL on the 2HS from cv.Triton (Wagner et al. 2008). SSR markers, Bmac0209/Falc666 and MWG680 are linked to the Rrs1 gene on the 3H from the Ethiopian landrace Abyssinian (Grønnerød et al. 2002) and cv.Triton (Graner and Tekauz 1996), respectively. SSR markers HVM60 and HVM36b show linkage to the Rrs4 on 3HL from Nigrinudum (Patil et al. 2003). Genger et al. (2003) developed a sequence characterized amplified region (SCAR) marker, HVS3 within one cM from Rrs1BC240 derived from a Turkish wild barley accession of *H. vulgare* ssp. *spontaneum*. More recently, researchers have developed cleaved amplified polymorphic sequences (CAPS) markers including 110315-AciI developed for the Rrs1-Rh4 derived from a Spanish landrace line (Hofmann et al. 2013). The restriction fragment length polymorphism (RFLP), Cxp3, was linked to Rrs13 on the 6H derived from *H. v.* ssp. *spontaneum* (Abbott et al. 1995). RFLP markers CDO545 and cMWG2018 were linked to Rrs2 on the 7HS from Atlas (Schweizer et al. 1995) and then Hanemann et al. (2009) fine mapped the Rrs2 (Rrs2Atlas) and identified three CAPS markers. While, Genger et al. (2005) linked the SSR marker HVM49 to Rrs15 on 7HL derived from *H. v.* ssp. *spontaneum*.

Marker assisted selection (MAS) analysis using marker systems such as RFLPs and SSR are time consuming, labor intensive and often loosely linked to the QTL, while next generation sequencing (NGS) has drastically reduced the cost and time of sequencing as well as SNP discovery. NGS has also led to the development of high-throughput SNP genotyping platforms, allowing researchers to better map QTL regions, and identify SNP markers that are more predictive of scald resistance. SNP markers are also better suited for high-speed genotyping and therefore offer breeders new methods for MAS that are faster, less expensive and more accurate. In order to improve the durability of scald resistance in Canadian barley cultivars it is important to identify new sources of barley scald resistance.

A previous study showed that cv. Seebe was resistant to *R. commune* (Xi et al. 2003a, b). Unpublished data on cv. Shyri by Luis Marquez-Cedillo et al., deposited in the GrainGenes database (wheat.pw.usda.gov/ggpages/SxG/) reported scald resistance QTLs on the 3H, 6H and 7H. As the use of genetic resistance is a desirable management strategy for controlling scald in barley (Singh et al. 2003), more reliable SNPs markers are needed for barley breeders. The aim of this study is to identify SNP markers for multiple leaf-scald resistance QTLs in cv. Seebe and cv. Shyri. For this study, we have phenotyped scald resistance on two RIL populations with each resistant parent crossed with susceptible cv. Harrington and genotyped them through RADseq analysis.

Materials and methods

Plant and fungal materials

We used two genetic populations created by F2-derived single-seed decent and each consisting of 150 F7 RILs. Descriptions of parental varieties are provided in Table 1. Four *R. commune* isolates (from central Alberta, Canada) were used in our study: Earl, E97-2, H97-2 and 40NROT01. Isolates Earl, E97-2 and H97-2 were provided by Dr. K. Xi (Alberta Agriculture and Forestry) while isolate 40NROT01 was provided by the Lacombe Agriculture and Agri-Food Canada centre.

Scald resistance assessment

Adult plant resistance (APR) in T98088 was assessed in two field locations (Lacombe and Edmonton, AB) in 2012 using isolate H97-2 and E97-2 respectively. Barley lines were planted as hill plots (8–10 seeds) in a randomized block design with two replications, and plots were spore-inoculated in mid-June and irrigated as required using sprinklers after inoculation. Scald severity was assessed in early August using a 0–9 rating scale, where nine represents maximum susceptibility in diseased leaf area (Couture 1980). Population T98089 was assessed for APR in 2015 in Lacombe using scald isolate 40NROT01 with the same methods. Scald seedling resistance (SR) was accessed in the greenhouses at Lacombe for both populations with isolate Earl. The experiment was laid out in a randomized block design with four replications, and disease was scored as a percentage of symptoms on 2nd and 3rd leaves, using a 0–3 scale according to Jorgensen and Smedegaard-Petersen (1995). Analysis of variance (ANOVA) was performed for field resistance assessment of both populations using SAS 9.4 (Statistical Analysis System, SAS institute), and broad-sense heritability was calculated using formula $h2 = \frac{Vg}{Vp}$ where $Vp = Vg + Ve + coV(g, location)$ for T98088 and $Vp = Vg + Ve$ for T98089.

RADseq analysis

Eighty randomly selected lines from population T98088 and 82 randomly selected lines from population T98089 were sequenced. Genomic DNA was extracted from seedling leaves using a DNA isolation kit (Norgen Biotek, Thorold, ON, Canada). DNA concentration was quantified using a Qubit 2.0 Fluorometer and Qubit dsDNA BR Assay kit (Life Technologies, Carlsbad, CA, USA) and normalized to 20 ng/μl. RADseq libraries were constructed as previously described (Mascher et al. 2013) except that we pooled amplified libraries from every 10–12 lines for size-selection ranging from 200–300 bp and 300–500 bp using a 2% E-gel system (Life Technologies). Size-selected libraries were then quantified using an Ion library quantification kit (Life Technologies) and normalized to 26 pM. Thereafter two pools of the same-size libraries (with or without parental libraries) were templated on Ion sphere particles (ISPs) by emulsion PCR using an Ion PGM Hi-Q OT2 kit on an Ion OneTouch2 machine (Life Technologies) per the manufacturer's instruction. Following the emulsion PCR templated ISPs were enriched on an Ion OneTouch ES (Life Technologies) for sequencing. A total of eight sequencing runs were performed on an Ion Torrent PGM system (Life Technologies) using the Ion PGM 200 Seq Kit and 318 v2 chips (Life Technologies) for each population.

Table 1 Parental pedigrees and scald ratings

Parent	Pedigree	Type	Source	Scald rating Field[a]
Harrington	Klages/3/Gazelle/Betzes//Centennial	2R, malt	Harvey and Rossnagel (1984)	4.5
Seebe	Masurca//Muller/Heydla	2R, feed	Helm et al. (1996)	2.9
Shyri	Lignee 640/Kober//Teran 78	2R, feed	Vivar and McNab (2001)	1.0

[a]Field ratings was assessed using a 0–9 rating scale, where 9 represents maximum susceptibility

DArT, SSR/EST, SCAR and CAPS marker analysis

Diversity arrays technology (DArT) markers on Harrington/Seebe RILs were obtained via commercial service from Diversity Arrays Technology Pty Limited, Australia. SSR and expressed sequence tag (EST) markers were examined by standard PCR in a 10 µl volume reaction containing 1 × Tsg reaction buffer (Bio Basic Inc., Markham, ON), 2 mM $MgSO_4$, 250 µM dNTPs, 0.5 µM each primer, 10 ng of DNA template, and 0.4 U of Tsg polymerase (Bio Basic). Amplification programs consisted of 3 min at 94 °C for denaturation, followed by 32 cycles of 94 °C for 45 s, 48–65 °C for 45 s, 68 °C for 1–1.5 min. PCR products were separated on 2% agarose gel. The CAPS marker 110315-AciI for Rrs1Rh4 was examined according to Hofmann et al. (2013) while SCAR marker HVS3 for Rrs1BC240 was examined according to Genger et al. (2003).

Sequence data analysis, QTL mapping and putative marker identification

The software TASSEL-GBS v4.3 (Glaubitz et al. 2014) with the Morex barley reference genome (assembly 082214v1, GCA_000326085.1) was used to initially identify SNPs from sequencing data. Later we updated sequence tags to match the most recent barley sequence (Hv_IBSC_PGSB_v2-January 2018). Tags (64 bp sequence reads) occurring at least ten times in the sequence data were aligned to the reference genome using BWA v0.7.12 (Li and Durbin 2009) before SNPs were called by TASSEL. The resulting data set was filtered to only lines, with no missing data at 90% or more sites (-mnTCov 0.1). SNPs which were called in less than 75% of the lines (-mnSCov 0.75) were also removed.

Following SNP identification, single marker analysis (SMA) was used to identify those linked to scald resistance using WinQTLCart 2.5 (Wang et al. 2011) and MapDisto1.7.5 (Lorieux 2012). Basic local alignment search tool (BLASTn) was conducted against the IBSC assembly_WGSMorex, assembly_WGSBowman databases (http://webblast.ipk-gatersleben.de/barley/) (e-value < 0.01), Ensembl Plants *Hordeum vulgare* (Hv_IBSC_PGSB_v2) (http://plants.ensembl.org/Hordeum_vulgare/Info/Index) Bolser et al. (2016) to anchor their physical locations and to search for overlapping genes to the SNP markers linked to scald resistance. Flanking sequences of SNPs were anchored to the reference genome if they shared 99–100% homology. Inclusive composite interval mapping (ICIM) was conducted using QTL IciMapping (ICIM) software (Wang et al. 2014a) using DArT, SSR and RADseq SNP markers. A LOD threshold of 2.5 and 1000 permutation tests were used to identify significant QTLs. Precise QTL maps are not expected and as the analyses were based on 80 or 82 lines.

SNP validation and conversion to allele specific PCR marker

Major SNP markers were verified by Sanger sequencing (Sanger et al. 1977). Specific primers to target flanking SNP sequences were designed using the online tool Primer3Plus and the SNP sequence reads (extended in length according to Morex genome reference). For each SNP target, six PCR reactions were pooled (50 µl volume) and cleaned using a PCR purification kit (Norgen Biotek), followed by size-selection of the target PCR amplicon using a 2% E-gel system (Life Technologies). Sequencing reactions were prepared using GenomeLab DTCS quick start kit (Beckman Coulter, Carlsbad, CA) with either the reverse or forward PCR primer, and then analyzed on a CEQ 8000 Genetic Analysis System (Beckman Coulter) as per manufacturer's instructions.

A SNP marker closest to the major 6H QTL was converted to an allele specific PCR marker according to Fechter et al. (2010). Forward primers matched the original sequence from Seebe except for the third base counted from the SNP at 3′ end. Reverse primers were designed using Primer3plus. The allele specific forward primer 6H-1119F was 5′-GCGTGACAAAT GTTTGCTTC-3′ and the allele specific reverse primer 6H1119R: 5′-CATCTGGACTTAAAACCCG AAA-3′.

Allele specific PCR was performed in a 10 µl volume containing 1 × Tsg reaction buffer (Bio Basic), 2 mM MgSO4, 200 µM dNTPs, 0.5 µM each primer, 10 ng of DNA template, 0.4 U of Tsg polymerase. PCR programs consisted of 3 min at 94 °C, followed by one cycle of touchdown 60 starting at 64 °C and 30 cycles of 94 °C for 45 s, 60 °C for 45 s, 68 °C for 1 min. PCR product was separated in 2% agarose gel, and photographed with AlphaImager EP (Alpha Innotech, San Leandro, CA, USA).

Results

Scald severity assessment

Greenhouse SR screening to scald isolate Earl, resulted in an intermediate to susceptible reaction in variety Harrington and a moderately resistant or resistant reaction for parents Seebe and Shyri (Table 2). The frequency distribution of scald seedling severity within the population T98089 was normal (Fig. 1). However, the more scald resistant population (T98088) had low susceptibility to isolate Earl, and produced a skewed frequency distribution of seedling severity (Fig. 1). APR to scald isolate 40NROT01 observed in parental and genetic population lines are summarized in Table 2. The results show significant differences in APR between the susceptible parent Harrington and the resistant parents Seebe and Shyri across the disease screening trials. In both, 2012 in Edmonton and 2015 in Lacombe, APR screening test results produced a normal frequency distribution (Fig. 1). In 2015, the mean field rating of Seebe was 2.66 ± 1.34, while the mean rating for Harrington was 5.72 ± 0.72. In 2012, the mean field ratings for Seebe and Shyri were 2.33 ± 0.67 to 4.0 ± 1.0 respectively, while the ratings on the susceptible Harrington checks ranged from 5.16 ± 1.56 to 8.5 ± 0.5. ANOVA analysis revealed significant genetic variation of scald resistance (Pr > F = 0.001), and interaction between genotype is insignificant (Pr > F = 0.501). Broad sense heritability R^2 was 0.726 from population T98089 and 0.538 from population T98088.

Sequencing data analysis, QTL mapping and SNP sequences verification

RAD sequencing of DNA libraries generated by a combination of *Pst*I and *Msp*I restriction enzymes was applied to find potential SNPs for scald resistance. Each of the RAD sequencing runs on 20/22 lines produced 3.7–5.38 million reads and the median read length varied from 138 to 237 bp. Sequencing data analysis using TASSEL-GBS v4.3 revealed a total of 2973 SNPs within the 80 selected lines from Seebe/Shyri population and 1230 SNPs within the 82 lines from Harrington/Seebe population. The sequence data flanking about 350 different SNP and DArT markers with significant LOD scores (> 5) from SMA were aligned by BLASTn and referenced to the barley genome sequence assembly map from Ensembl Plants (Hv_IBSC_PGSB_v2). Many of the SNP markers with the highest LOD scores were anchored to the barley physical map in the same approximate physical locations as SNP markers identified by ICIM analysis (results are summarized in Table S2). We then focused further comparative analysis on SNP markers identified by the two QTL analysis techniques and overlapping on the barley physical map (Hv_IBSC_PGSB_v2).

ICIM analysis of the sequenced lines within the Seebe/Shyri population, identified three APR QTLs on 3H, 4H, and 5H attributable to Shyri, and two additional APR QTLs on 2H and 6H attributable to Seebe (Fig. 2). The identified Qsc3H-Shyri QTL was tightly linked to SNP marker chr3H_498944660 and likely represents the previously identified Rrs1 QTL. While the strongest linkages identified by SMA to SR

Table 2 Basic statistics of scald severity in lines of Seebe/Shyri and Harrington/Seebe populations

Test[a]	Mean of parents			RIL lines			Isolate	Population
	Seebe	Shyri	Harrington	Min	Max	Mean		
2003 Lac	0.125 ± 0.13	0.05 ± 0.05	1.85 ± 0.40**	0	2.74	0.87 ± 0.26	Earl	Harrington/Seebe
2003 Lac	0.125 ± 0.13	0.05 ± .05	1.85 ± 0.40**	0	2.37	0.16 ± 0.07	Earl	Seebe/Shyri
2012 Edm	3.50 ± 0.67	4.0 ± 1.0	8.50 ± 0.50**	1	9	3.97 ± 0.61	E97-2	Seebe/Shyri
2012 Lac	2.33 ± 0.67	3.0 ± 1.0	5.16 ± 1.56**	0	6	2.68 ± 0.60	H97-2	Seebe/Shyri
2015 Lac	2.66 ± 1.34	–	5.72 ± 0.72**	0	8	4.27 ± 0.62	40NROT01	Harrington/Seebe

Lac lacombe, *Edm* Edmonton

[a]SR was evaluated in 2003 for two populations; APR was assessed in 2012 and 2015

**Significant difference between susceptible Harrington and resistant Seebe or Shyri according to *t* test ($P < 0.02$)

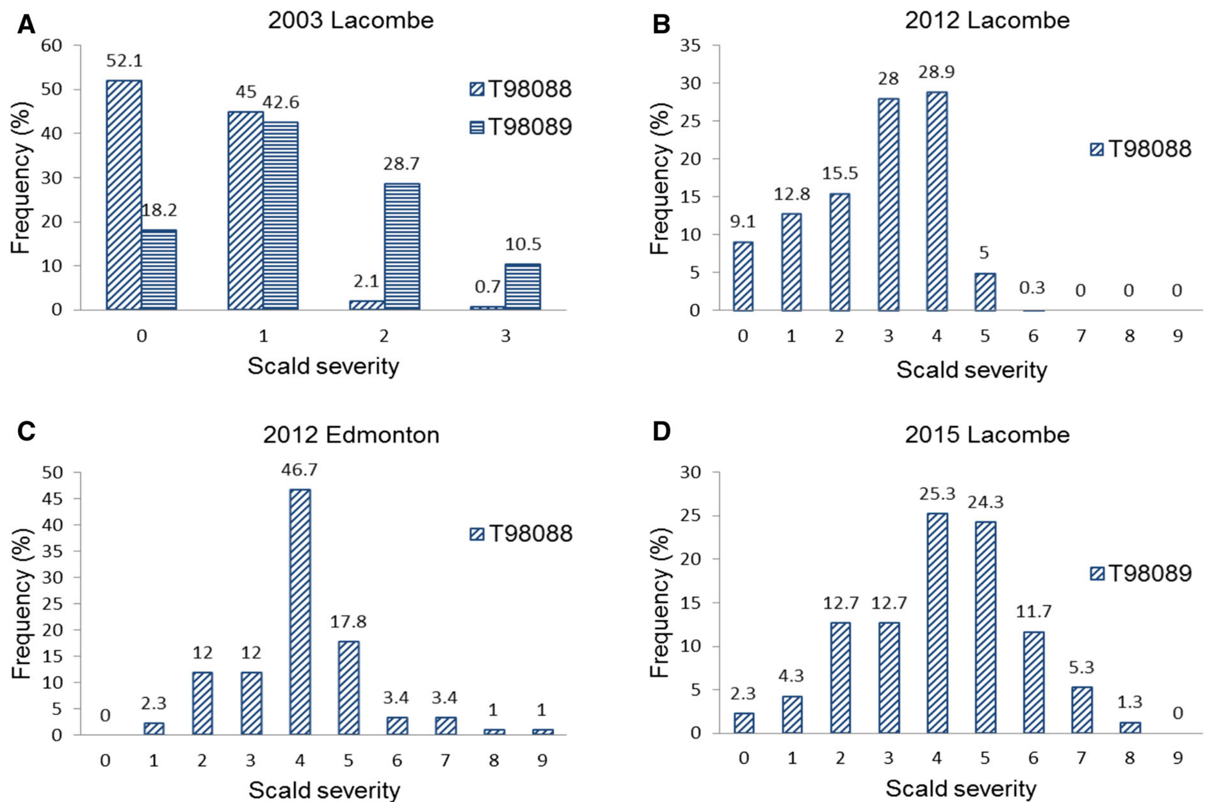

Fig. 1 Frequency distribution of scald severity from multiple tests on Seebe/Shyri (T98088) and Harrington/Seebe (T98089) populations. **a** The two populations were inoculated with isolate Earl for SR assessment; **b–d** isolate H97-2, E97-2 or 40NROT01 was used respectively for APR evaluation

were observed between SNP markers chr3H_498136366 and chr3H_498944660 (Table S2), which overlap three genes HORVU3Hr1G065350, HORVU3Hr1G065360 and HORVU3Hr1G065510, respectively. QTL Qsc2.6H was associated with up to 70.9% of phenotypic variation for APR by ICIM analysis (Table 3) and SNP markers chr6H_11180130 and chr6H_13203831 were most predictive of SR and APR resistance from Seebe. The two QTLs discovered by ICIM were confirmed by SMA and BLASTn alignment near the 6HS telomere in both populations. SNP markers chr6H_12318331 and chr6H_13203831 (Table S2) overlapped genes HORVU6Hr1G005540 and HORVU6Hr1G005990 with each gene, encoding a putative plant peroxidase gene and a transcription initiation factor, respectively. Marker chr6H_7681197 overlapped gene HORVU6Hr1G002980 with protein motifs consistent with a mitochondrial/chloroplastic transcription termination factor. Marker chr6H_8562850 overlapped gene HORVU6Hr1G003940 encoding a putative serine–threonine/tyrosine–protein kinase gene, while our most strongly linked SNP marker to scald disease resistance chr6H_11180130 did not overlap any barley gene coding regions. Also of note, ICIM analysis of sequenced lines from the Harrington/Seebe population also revealed two more QTLs on the 6H contributing to both SR and APR.

Smaller affect QTLs were also identified via ICIM analysis as contributing 13–23% of APR scald resistance on the 2HL, 4HL and 5HL. The Qsc2H-Seebe QTL linked to SNP marker chr2H_543512242 associated with 23% APR variation in the Seebe/Shyri population. Linked marker chr2H_543448499 overlapped gene HORVU2Hr1G075420 an unknown protein with an RNA recognition motif while further analysis by SMA also identified SNP markers aligned on the 2HL to SR in both the Harrington/Seebe and Seebe/Shyri population. On the 4H, a 16 Mb region was found to contribute about 13% APR, Qsc4H-Shyri QTL and was linked to SNP markers chr4H_60268307and chr4H_60268310. The flanking DNA SNPs sequences were located on the long arm of

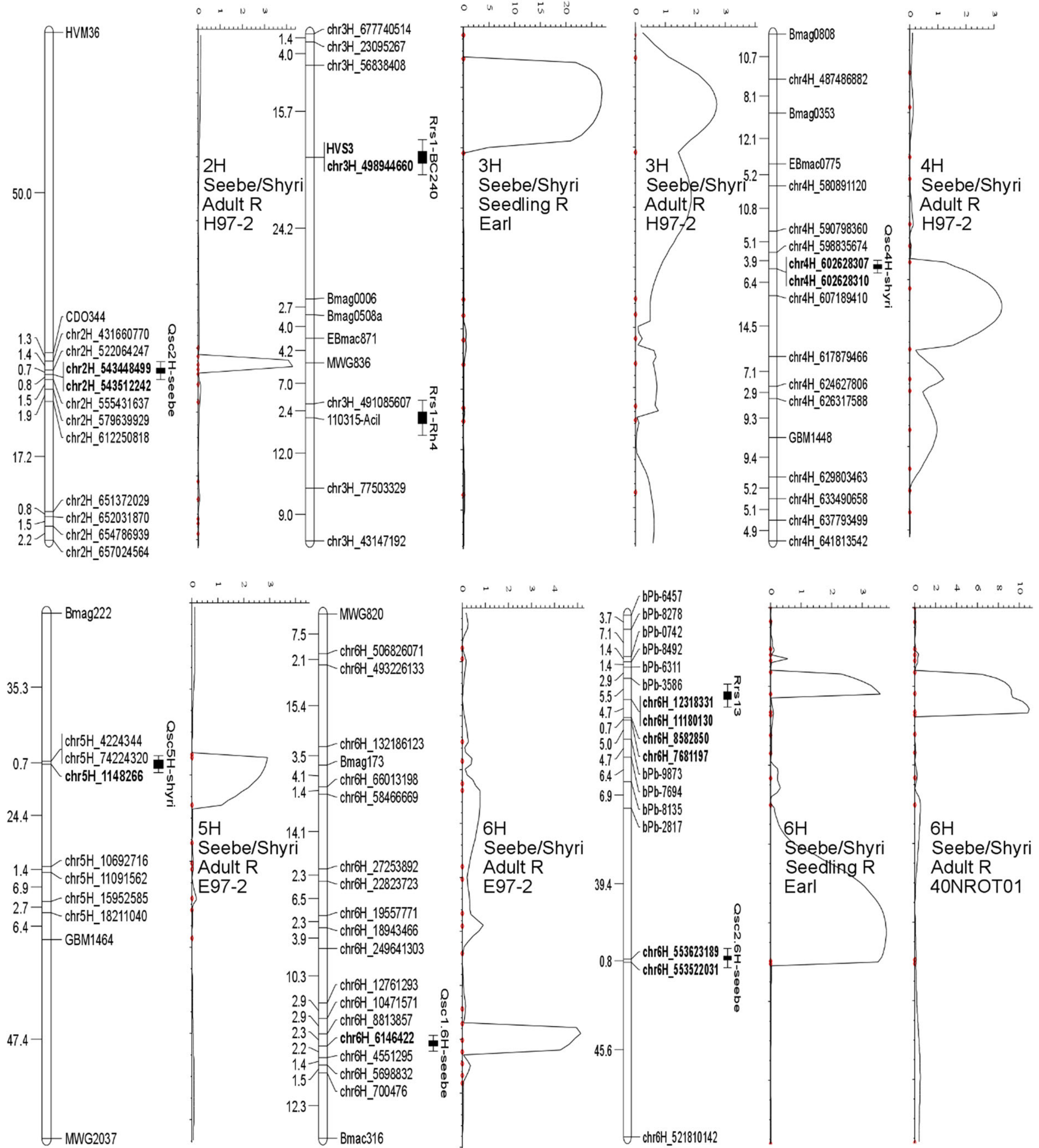

Fig. 2 QTLs for resistance to *R. commune* identified by inclusive composite interval mapping (ICIM) using SNPs and scald severity data from multiple experiments. The genetic distances are shown in centiMorgans (cM), and the marker maps were redrawn using MapChart developed by Voorrips (2002). Seedling resistance was evaluated in 2003 for both populations inoculated with isolate Earl, Seebe/Shyri population was evaluated for APR in 2012 with isolates H97-2 and E97-2, Harrington/Seebe population was evaluated for APR in 2015 with isolate 40NROT01

chromosome 4H and found to overlap gene HORVU4Hr1G077060 containing protein ABC transporter-like motifs. Marker chr4H_607189411 located only 4561 Kb from SNP chrH4_602628310, was also identified by SMA associated to both APR in the Seebe/Shyri population and SR resistance in the

Table 3 Markers and QTLs for scald resistance revealed by RAD sequencing and ICIM analysis

Marker ID	QTLs	SNP (R SNP)[a]	R^2 (%)[b] APR/SR	Correlation[c] APR/SR	P[d]	Population[e]	Isolate[f]
chr2H_543512242	Qsc2H-Seebe	G/A (G)[s]	23.2/0.8	− 46.7/− 2.9	0.00	T98088	H97-2
chr2H_543448499	Qsc2H-Seebe	T/C (T)[s]	16.1/1.14	− 37.1/− 10.7	0.00	T98088	H97-2
chr3H_498944660	Rrs1	G/T (T)[sh]	6.6/23.7	− 25.7/− 48.7	0.00	T98088	H97-2/Earl
chrH4_602628307	Qsc4H-Shyri	T/C (C)[sh]	13.1/2.16	− 27.6/− 14.7	0.01	T98088	H97-2
chrH4_602628310	Qsc4H-Shyri	T/G (G)[sh]	13.1/2.16	− 33.9/− 14.7	0.01	T98088	H97-2
chr5H_1148266	Qsc5H-Shyri	C/T (T)[sh]	15.3/0.01	− 37.5/− 0.72	0.00	T98088	E97-2
chr6H_6146622	Qsc1.6H-Seebe	T/G (T)[s]	13.2/0.42	− 41.5/− 6.55	0.00	T98088	E97-2
chr6H_8562850	Qsc2.6H-Seebe	G/C (G)[s]	64.6/10.1	− 82.8/− 31.9	0.00	T98089	40NROT01
chr6H_7681197	Qsc2.6H-Seebe	C/A (C)[s]	64.6/10.7	− 82.8/− 32.8	0.00	T98089	40NROT01
bPb-6311	Qsc2.6H-Seebe	–	45.8/9.6	− 67.7/− 31.0	0.00	T98089	40NROT01
chr6H_11180130	Qsc2.6H-Seebe	C/T (C)[s]	70.4/16.0	− 83.9/− 43.3	0.00	T98089	40NROT01
chr6H_12318331	Qsc2.6H-Seebe	G/T (G)[s]	70.9/13.8	− 84.2/− 36.36	0.00	T98089	40NROT01
chr6H_553522031	Qsc3.6H-Seebe	C/T (C)[s]	5.88/17	− 7.67/− 42.8	0.00	T98089	Earl

[a]Resistance allele is in bracket, superscript s stands for Seebe allele, superscript sh stands for Shyri allele

[b]Phenotypic variation within the sequenced lines explained by a single QTL linked to the SNP. *APR* adult plant resistance, *SR* seedling resistance

[c]Correlation of SNP markers to scald severity ratings of the sequenced lines

[d]Probability that the marker is not linked to scald resistance

[e]T98088 RIL cross Seebe/Shyri, T98089 RIL cross Harrington/Seebe

[f]Scald isolate used for inoculation

Harrington/Seebe. Finally, Qsc5H-shyri QTL attributed about 15% of APR. SNP marker chr5H_1148266 from parent Shyri, was detected linked to the QTL by both ICIM and SMA in the Seebe/Shyri population. A 3651 kb region in the telomeric region of 5H demonstrated several more SNP markers linked to APR and SR by SMA (Table S2). SNP marker chr5H_2257577 identified by SMA was linked to both SR and APR resistance and overlapped gene HORVU5Hr1G000590, a putative signal transduction histidine kinase-related protein. While marker chr5H_3766117 was linked to APR in Edmonton by SMA and overlapped gene HORVU5Hr1G001090, with protein motifs characteristic of a peroxidase gene.

Conversion of a major 6H QTL SNP marker into an allele specific PCR marker

PCR tests were carried out with the allele-specific marker chr6H_11180130 on the sequenced lines of the Harrington/Seebe population, T980089. About 91% of the lines that showed the marker band were resistant to scald according to the 2015 scald severity rating. Genotyping of the sequenced lines with the allele-specific marker matched 97.5% of the results from Ion Torrent PGM sequencing. The profile of PCR marker band in 2% agarose gel is shown in Fig. 3.

Discussion

By using RADseq on an NGS system, we were able to identify seven scald resistance QTLs allocated on five chromosomes (Fig. 2, Table 2). One of the benefits of utilizing NGS based methods to genotype both genetic and breeding plant populations is the generation of valuable sequence data along with the SNP marker calls. ICIM and SMA analysis followed by BLASTn searches referenced to the barley physical map (Ensembl Plants) helped us confirm map locations and served to better anchor SNP markers. We were then able to use various bioinformatic resources to compare sequence data and identify putative candidate genes that may affect scald resistance at these QTLs. The RADseq method generated a greater number of

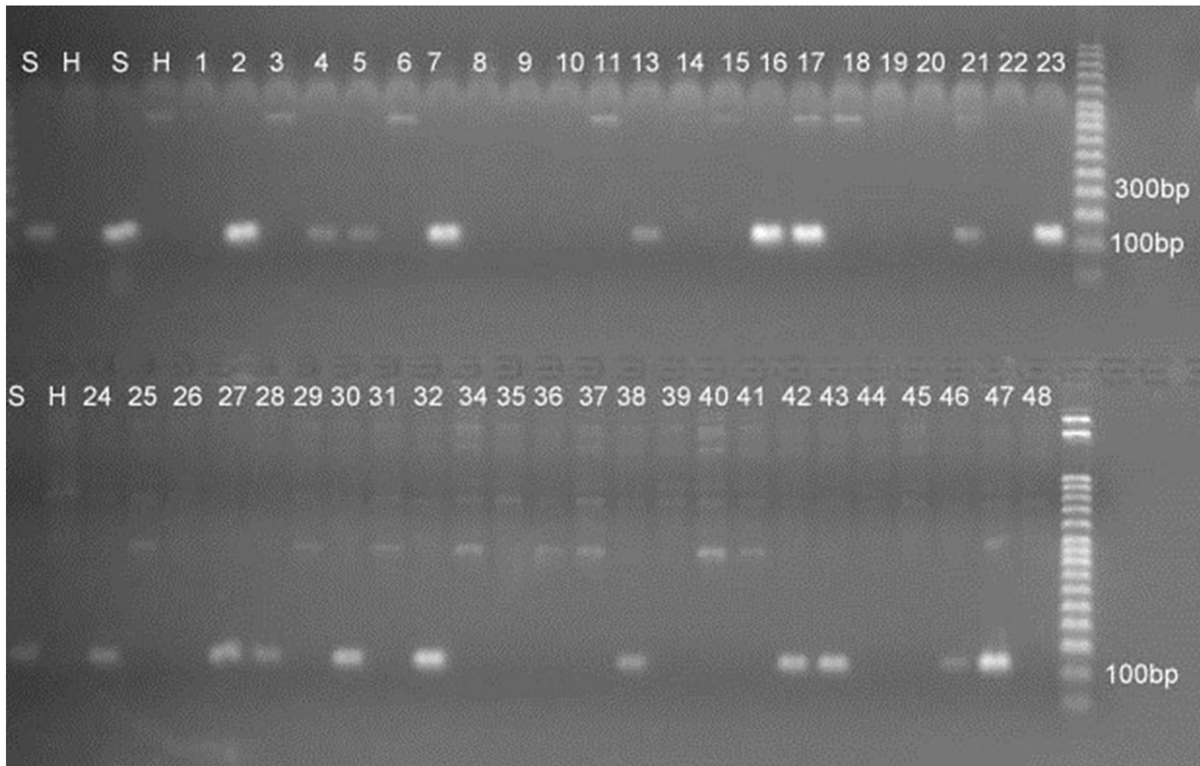

Fig. 3 Allele-specific marker 6H_11180130 tested on lines from the Harrington/Seebe population and shown in a 2% agarose gel. *S* Seebe, *H* Harrington. Marker band = 120 bp. Genotyping with the PCR marker on sequenced lines matched 97.5% of the results from the Ion Torrent PGM sequencing

usable polymorphic SNP markers when compared to the DArT analysis. For example we were able to identify within the Harrington/Seebe population 1230 polymorphic SNPs by RADseq while the DArT analysis only generated about 450 polymorphic SNPs. Overall, our RADseq analysis of our two RIL populations was able to identify SNP markers linked to several scald resistance QTLs.

QTL analysis of the Seebe/Shyri population revealed a 3H QTL linked to both SR and APR (Fig. 2), and the marker chr3H_498944660 for this QTL overlapped with Rrs1BC240 reported by Genger et al. (2003). BLASTn search indicated that marker chr3H_498944660 is located within the gene HORVU3Hr1G065510 which contains a conserved SWAP/Surp (Suppressor-of-White-APricot) protein domain. Whether this protein is involved in a resistance mechanism remains a question for further study. In addition, the marker 11_0315 for Rrs1Rh4 on chromosome 3H reported by Hofmann et al. (2013) did not identify scald resistance by ICIM analysis (Fig. 2). Which is further proof that the previously named Rrs1 QTL involves different alleles that offer pathotype-specific resistance as reported by Bjørnstad et al. (2002). We did however identify significantly linked SNP markers within the Rrs1Rh4 QTL region by SMA (summarized in Table S2). Genes identified overlapping significant SNP markers within the proposed Rrs1 region appear to represent clusters of putative resistance related genes involved in signal perception, transduction, gene regulation and expression including disease resistance related genes, such as leucine rich repeat (LLR), concanavalin A-like lectin/glucanase, wall-associated receptor kinase galacturonan-binding domain, and ubiquitin-fold (Krattinger and Keller 2016).

A second major QTL, Qsc2.6H-Seebe identified by ICIM and SMA on the 6HS telomeric region explained up to 70% of the phenotypic variation for APR within the sequenced lines (Fig. 2, Tables 3, 4) from the Harrington/Seebe population. The four SNP markers linked to this QTL, chr6H_7681197, chr6H_8562850,

chr6H_11180130 and chr6H_13203831, overlapped genes with functions often related to disease resistance, including protein kinases, transcription factors, and peroxidases (Table S2). Marker chr6H_7681197 was found overlapping genes with transcription factor-like motifs, while marker chr6H_12318331 overlapped gene HORVU6Hr1G005540, a putative secretory plant peroxidase gene.

In addition to the major QTLs on the 3H (Rrs1) and 6H (Qsc2.6H-Seebe, Qsc1.6H-Seebe), ICIM mapping found additional QTLs on the 2H (Qsc.2H-Seebe), the 4H (Qsc.4H-Shyri), the 5H (Qsc.5H-Shyri) and the 6H (Qsc3.6H-Seebe), that were also confirmed by SMA (Table S2). In the Harrington/Seebe population, additional SNP markers were found linked to both SR and APR on the 2HL. The most significant SNP markers linked to seedling resistance were chr2H_613632775, chr2H_758717415, chr2H_760246167, chr2H_76046095 and chr2H_767502448 by SMA (Table S2). We also found the physical map locations of these SNPs overlapping putative candidate genes for disease resistance including yet another possible secretory peroxidase and a HAD-superfamily hydrolase (phosphohydrolases possibly involved in detoxification of phosphorylated compounds, Kuznetsova et al. 2015). QTL Qsc.4H-Shyri, linked to APR in the Seebe/Shyri population (Fig. 2) and SR in the Harrington/Seebe population was found linked to SNP markers, chrH4_602628307 and chrH4_602628310. Both markers overlapped an ABC transporter type 1-like gene, HORVU4Hr1G077060. ABC transporters are a large family of proteins involved in the transport of a wide variety of different compounds, like sugars, ions, peptides, and more complex organic molecules. Many studies have shown that ABC transporters transport secondary metabolites and have a role in withstanding and hindering plant pathogens (Hwang et al. 2016; Krattinger et al. 2009). Another gene of note, HORVU4Hr1G073610 a possible pectin esterase gene was found to overlap SNP marker chr4H_590798360 identified linked to scald resistance in both populations by SMA (Table S2). Previous research by Marzin et al. (2016) suggested a pectin esterase inhibitor (PEI) genes were involved in mediating resistance to *R. commune* in barley. They found a family of putative PEI genes in the genomic region co-segregating with the resistance gene Rrs2 on the 7H. The role of PMEs in plants have been

Table 4 A comparison table of barley physical map locations of identified SNP markers for scald resistance by ICIM analysis and their corresponding overlapping genes

SNP ID	QTL	Barley genes[a]	Protein function/domain[b]
chr2H_543512242	Qsc2H-Seebe	–	–
chr3H_498944660	Rrs1	HORVU3Hr1G065510	SWAP (Suppressor-of-White-APricot)/surp domain-containing protein
chrH4_602628307	Qsc4H-Shyri	HORVU4Hr1G077060	ABC transporter type 1, transmembrane domain/AAA + ATPase domain/P-loop containing nucleoside triphosphate hydrolase
chr5H_1148266	Qsc5H-Shyri	–	–
chr6H_6146622	Qsc1.6H-Seebe	HORVU6Hr1G002240	RNA recognition motif domain
chr6H_8562850	Qsc2.6H-Seebe	HORVU6Hr1G003940	protein kinase, Serine–threonine/tyrosine–protein kinase, catalytic domain
chr6H_7681197	Qsc2.6H-Seebe	HORVU6Hr1G002980	Transcription termination factor, mitochondrial/chloroplastic
chr6H_11180130	Qsc2.6H-Seebe	–	–
chr6H_12318331	Qsc2.6H-Seebe	HORVU6Hr1G005540	Haem peroxidase, plant/fungal/bacterial, Peroxidases heam-ligand binding site/Plant peroxidase/Secretory peroxidase
chr6H_553522031	Qsc3.6H-Seebe	–	–

[a]SNP locations were identified by BLASTn analysis at EnsemblPlants (Bolser et al. 2016) aligned to *Hordeum vulgare* genome sequence (Hv_IBSC_PGSB_v2)

[b]Protein function/domain encoded by the genes provided at EnsemblPlants data base release 38 (January 2018) http://plants.ensembl.org/Hordeum_vulgare/Info/Index

implicated in several processes, including cell wall extension a known mechanism to physically block pathogen spread. However, Marzin et al. (2016) were unable to determine or characterize PEI involvement in mediating resistance at the Rrs2 QTL to the scald pathogen by complementation and overexpression analysis. The Qsc5H-Shyri QTL identified by ICIM was linked SNP marker chr5H_1148266 for APR resistance and did not overlap any genes. However, SMA identified markers chr5H_2257608 and chr5H_2257577 also located near the Qsc5H-Shyri QTL overlapped gene HORVU5Hr1G000590, a putative signal transduction histidine kinase-related protein kinase. Several markers were identified by SMA in the telomeric region of 5H short arm, and some overlapped genes with plant resistance protein (R-protein) function commonly referred to as a nucleotide binding site-leucine rich repeat (NBS-LRR; reviewed by Urbach and Ausubel 2017).

In summary, the components involved in resistance to leaf scald in Seebe and Shyri have been elucidated by RADseq, QTL mapping and comparative genomic study. QTLs for scald resistance were found on all chromosomes except the 1H and 7H chromosomes. However some of these QTLs identified in our study, are likely scald isolate-specific. QTLs, Qsc2H-Seebe, Qsc4H-Shyri, Qsc5H-Shyri, Qsc1.6H-Seebe, and Qsc3.6H-Seebe revealed levels of resistance that varied for APR and SR between location and with different scald isolates. However, the major QTL Qsc2.6H-Seebe attributable to Seebe and the Rrs1 on the 3HL in Shyri were found to improve resistance to all scald isolates tested and therefore these newly identified linked SNPs could be utilized for the application of MAS to pyramid more durable scald resistance into new varieties.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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